

Appendix A
Pending Claims 22627-20014.03

1. (Amended) A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

(a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter;

(b) replicating the cell of step (a) to produce a population of cells; and

(c) introducing a helper virus to the population of cells of step (b);

(d) wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

2. A method according to claim 1, wherein said helper virus is an adenovirus.

3. (Amended) A method according to claim 1, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

4. A method according to claim 1, wherein said mammalian cell of step (a) comprises the combined rep and cap genes of AAV in which the p5 promoter has been replaced by a heterologous promoter.

5. A method according to claim 4, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

6. (Amended) A cell produced by the method of claim 1, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

7. (Amended) A cell produced by the method of claim 3, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

8. (Amended) A cell produced by the method of claim 4, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

9. (Amended) A cell produced by the method of claim 5, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

10. (Amended) A mammalian cell for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a promoter, and a stably integrated rep gene operably linked to a helper virus-inducible heterologous promoter; wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter and wherein said cell exhibits helper-virus-inducible expression of said stably integrated AAV rep gene.

11. (Amended) An AAV packaging cell of claim 10, wherein said helper-virus-inducible expression of said stably integrated AAV rep gene is inducible by adenovirus.

12. (Amended) An AAV packaging cell of claim 10, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

13. An AAV packaging cell of claim 10, wherein said cell comprises the combined rep and cap genes of AAV in which the p5 promoter has been replaced by a heterologous promoter.

14. An AAV packaging cell of claim 13, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

15. (Amended) An AAV packaging cell of claim 10, further comprising a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

16. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 10;

(b) introducing a recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;

(c) introducing a helper virus; and

(d) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

17. (Amended) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) providing an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest operably linked to a promoter;

(b) introducing a helper virus; and

(c) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

21. (Amended) A method of determining the infectious titer of an rAAV vector preparation, comprising the steps of:

(a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.

22. (New) The method of claim 1, further comprising the step of selecting a cell exhibiting helper-virus-inducible expression of said stably integrated AAV rep gene.